

Real-time chemical analysis of root filling materials with heating

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Real-time chemical analysis of root filling materials with heating; Guidelines for safe temperature levels.

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Real-time chemical analysis of root filling materials with heating; guidelines for safe temperature levels.

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Running Title: Heat’s effect on root fillings

Key words: calcium silicate; endodontic heat carriers; epoxy resin sealer; gutta-percha; Raman spectroscopy; warm obturation.

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Abstract:

Aim To investigate the chemical changes affecting different types of gutta-percha and endodontic sealers during heating, and correlate changes with the heating capacity of different heat carriers.

Methodology The heating capacity of three endodontic heat carriers were evaluated using thermocouples to produce heat profiles. The devices were activated at different temperature set-ups, in continuous or cut-out modes. Chemical changes of six brands of gutta-percha and four types of sealers were assessed in real-time during heating using micro-Raman spectroscopy equipped with a heating stage. Raman spectra of each tested material were averaged and compared at different temperature levels. The sealers were further assessed by Fourier transform infrared (FTIR) spectroscopy.

Results None of the tested heat carriers achieved the temperature levels that were set by the devices and recommended by the manufacturer. The use of continuous heating mode resulted in higher rises in temperature than the 4s cut-out mode that reached 110 °C. The various brands of gutta-percha exhibited different chemical changes in response to heat. Some changes even occurred below temperature levels generated by the heating devices. All sealers revealed changes in their chemical composition upon heating. Changes in epoxy resin- and zinc oxide eugenol-based sealers were detectable at 100 °C, with structural alterations beyond that temperature and irreversible changes after cooling. Water loss was irreversible in BioRoot, but its chemical structure was stable as well as for the TotalFill.

Conclusions The heating capacity of endodontic heat carriers needs to be standardized, so that the temperatures delivered by the tips is the same as that set on the dial. Practitioners should be aware of the actual temperatures generated by these devices, and the suitability of sealers to be used at the temperature levels achieved.

Introduction

Root canal filling aims to seal the root canal space after chemo-mechanical preparation to avoid microbial recontamination. For some time, gutta-percha has been the dominant root filling material (Ørstavik 2005). Many different techniques for using gutta-percha have been described, all of which involve using gutta-percha as a core material along with a flowable sealer to occupy unfilled spaces. Heat has been used to enhance the adaptation of solid gutta-percha within the complex root canal anatomy (Wu *et al.* 2002), in an attempt to improve the sealing quality of the root filling (Lea *et al.* 2005). During warm compaction, gutta-percha is heated inside the canal using heat carriers, which can be a hand instruments heated on a flame or an electronic device that generates heat and conveys it through metallic tips of various sizes.

Several studies have verified that the actual heat generated by carriers do not reach the set temperature on the equipment's dial (Silver *et al.* 1999, Venturi *et al.* 2002, Viapiana *et al.* 2015). This may be beneficial, as the high temperature levels recommended for warm compaction (180 °C - 200 °C) would be deleterious to the periodontium (Johnson *et al.* 2016). Heat may be further attenuated by the heat dissipation capability of teeth as well as endodontic sealers that further reduces the temperature conducted to surfaces of roots (Viapiana *et al.* 2014). This explains the minimal increase in the external root surface temperature by a few degrees above body temperature during heating (Venturi *et al.* 2002 Viapiana *et al.* 2015). Although some heat carriers were found to increase the root surface temperature up to 14.5 °C (Silver *et al.* 1999).

Regardless the widespread use of warm root filling techniques, the effect of heat on root filling materials has not been investigated extensively. Work by Schilder and colleagues (Schilder *et al.* 1974, Goodman *et al.* 1981, Schilder *et al.* 1985) reported the heat profiles of gutta-percha and temperatures of phase transformations with heating. It was established that gutta-percha was present in a β -phase below 49 °C that transformed into an α -phase between 53-59 °C, after which it became amorphous. Upon rapid cooling, the material transformed directly into a β -phase without intermediate formation of an α -phase and the material shrinks (Schilder *et al.* 1985). Differences in the thermal behaviour of different gutta-percha brands using differential scanning calorimetry has been reported

(Roberts *et al.* 2017). Roberts *et al.* (2017) reported that gutta-percha was stable during warm vertical compaction contrary to Maniglia-Ferreira *et al.* (2008) who suggested the break-down of gutta-percha's molecular structure.

The effect of heat on endodontic sealers is not well documented. A rise in the temperature to 100 °C has been reported to result in degradation of epoxy resin-based sealers and evaporation of water from calcium silicate-based sealers (Viapiana *et al.* 2014, Viapiana *et al.* 2015, Camilleri 2015, Atmeh & AlShwaimi 2017). The duration of heat application was also found to affect the stability of sealers (Atmeh & AlShwaimi 2017).

This study aims to correlate the heating capacity of several endodontic heating devices with changes in the chemical structure of different brands of gutta-percha and endodontic sealers upon heating.

From this data, recommendations for the range of temperature levels tolerated by root filling materials can be used with the devices tested during warm compaction techniques.

Materials and Methods

Heat profiles of endodontic carriers

Three endodontic heat carriers were tested: E&Q Master (Meta Biomed, Chalfont, PA, USA), SuperEndo B&L (B&L Biotech, Gyeonggido, Korea), and System-B (Sybrondental, Orange, CA, USA). The E&Q Master was supplied with fine/fine-medium heating tips and could be operated at 180 °C and 230 °C with automatic cut-out after 4 seconds. SuperEndo B&L was operated at 230 °C using tips with three tapers (0.06, 0.08, 0.10) and size 55 heating tip. System-B was set at 200 °C and heating tips with 0.06, 0.08, 0.10, or 0.12 tapers were used.

The temperature generated by each heat carrier was recorded using K-type thermocouples (2 mm diameter, Maplins, Birmingham, UK) at 2 mm, 8 mm and 16 mm from the tip of the carrier. The thermocouples were connected to a multi-channel data logger (PicoData Logger, TC-08, St Neots, UK) and fixed into a 3 mm thick Perspex plate to ensure reproducibility and intimate contact with the heating tip. Prior to measurements, the heating carriers were acclimatized to 37 °C (Hybaid Shake and Stack Oven, Thermoscientific, Loughborough, UK) (Fig.1). Temperatures were recorded in real-time at a rate of 1s⁻¹ for 112s using the PicoData logging software. The heat profiles generated by the devices were

measured over 3 cycles of heating (4 seconds) and cooling (30 seconds). Devices with continuous heating mode option (SuperEndo B&L and System-B) were activated for 30 seconds then left to cool. The measurements were repeated 3 times and their average was calculated and plotted.

Assessment of root filling materials during heating

Six brands of gutta-percha were tested; ProTaper Universal (PTU) and ProTaper Next (PTN) (Mailliefer Instrument Holding, Ballaigues, Switzerland), Wave One (WO) (Dentsply Mailliefer, Ballaigues, Switzerland), Wave One GOLD (WG) (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA), thermo-plasticized injectable gutta-percha (TPI), and 2% standardized points (STD) (Sure Dent Corp, Gyeonggi-do, Korea). The four sealers assessed were epoxy resin-based AH Plus (Dentsply International, Addlestone, UK), zinc oxide eugenol-based Pulp Canal Sealer (Kerr, Orange, CA, USA), and two calcium silicate-based TotalFill BC Sealer (FKG Dentaire, La Chaux-de-Fonds, Switzerland), and BioRoot RCS (Septodont, Saint-Maur-des-Fossés, France).

-Raman Spectroscopy

The materials were placed separately on a glass slide inside a heating chamber attached to a Surface Enhanced Raman Scattering (SERS) Spectroscope (LabRAM HR EVO, Horiba Scientific, Kyoto, Japan). Imaging was performed using a 688nm Helium Neon laser beam of 11 mW power with a 600 grooves/mm diffraction grating and a 50x/0.75 NA (numerical aperture) objective lens at room temperature. Gutta-percha samples were heated gradually from 30 °C to 200 °C at a 10 °C/min rate and the spectra acquired at 30, 40, 50, 60, 70, 80, 100, 150, and 200 °C temperatures. Sealers were heated to 200 °C at a rate of 30 °C/min and the spectra acquired at 25, 50, 75, 100, 125, 150, 175 and 200 °C temperatures. An additional spectrum (cooled) was acquired for each sample after cooling down to room temperature after 30 minutes.

Acquired Raman spectra were uploaded into spectral analysis software (SpectraGryph-1.0, available at www.effemm2.de/spectragryph/), and peaks of interest were determined and compared for each material at each temperature. To analyse changes in gutta-percha, the integrated intensity ratio was measured as a ratio between each peak's area to a reference peak (105 cm⁻¹) in the same spectrum. Both Raman peaks at 105 cm⁻¹ and 311 cm⁻¹, which represent barium sulfate and zinc oxide

respectively (Fig.2-d), were considered as internal standards due to their thermal stability within the temperature levels used in this study.

To identify irreversible changes, the integrated intensity ratios of peaks were plotted as percentages to the intensity of same peaks at 30 °C using the following formula:

$$\text{Percentage of Change} = \left(\frac{I_x - I_{30}}{I_{30}} \right) \times 100\%$$

Where (I_x) is the integrated intensity of the peak at 200 °C or after cooling, while (I_{30}) is the intensity of the same peak at 30 °C. The change after cooling was considered reversible if it did not exceed the range between the highest and lowest levels measured for the control peak (311 cm⁻¹) at any temperature in each material.

-Fourier Transform Infrared Spectroscopy

The sealers were also investigated using Fourier Transform Infrared (FT-IR) by attenuated total reflectance spectroscopy (FT-ATR). A smart MIRacle ATR accessory (ThermoFisher Scientific, Waltham, MA, USA) was attached to a Nicolet 6700 spectrometer (ThermoFisher Scientific, Waltham, Massachusetts, USA) with a liquid nitrogen cooled, high-speed, high sensitivity mercury cadmium telluride (MCT) detector. The standard diamond crystal plate was replaced with a MIRacle heated diamond plate attached to a control module (Pike Technologies, Fitchburg, WI, USA). The spectrometer was set to measure between 650 cm⁻¹ and 4000 cm⁻¹ at a resolution of 16, scans to average of 2 and data spacing of 1.928 cm⁻¹ at each measurement in absorbance mode. Background spectra were collected and stored at room temperature (21 ± 1 °C). Sealers were mixed and/or dispensed following manufacturers' instructions and were placed onto the ATR plate ensuring the diamond crystal (3 mm diameter) was positioned centrally within a ring 16 mm internal diameter. A baseline measurement was taken immediately after sample placement at room temperature (21 ± 1 °C) and then the heating module was activated to heat the samples at a rate of 12.5 °C/min. Measurements were taken from 25 °C to 200 °C at increments of 25 °C and then air-cooled to 25 °C and re-measured. All spectra were baseline corrected between 650-4000 cm⁻¹ and an average (n=3) was taken. The testing assembly was kept high relative humidity.

Results

Heat profiles of endodontic carriers

The heat profiles of tested heat carriers with different tips are shown in Fig.3. None of the devices reached the selected temperature shown on their display, which was below 60 °C in the 4s cut-out mode regardless of the temperature setting (Table-1). There was no specific pattern in the change of temperature in relation to the size, taper or location of the tips. The E&Q Master could only be operated in the cut-out mode and set at 180 °C (Fig.3-a) or 230 °C (Fig.3-b). Although the maximum temperature with the 230 °C selection was higher than with 180 °C, the actual temperature did not exceed 60 °C in both. The highest temperature generated by SuperEndo at 230 °C in cut-out mode was 60 °C (Fig.3-c) compared with 110 °C achieved in the continuous mode (Fig.3-d). System-B generated heat below the set level at 200 °C. The highest temperature was 55 °C in the cut-out mode (Fig.3-e) and 65 °C in the continuous mode (Fig.3-f).

Root filling materials and heating

- Gutta-percha

The Raman spectra of gutta-percha at 30 °C are shown in Fig.2-a, with peaks assignment in the Table in Fig.2-d. The percentage of change in the intensity of each peak after heating to 200 °C or cooling compared to peaks at room temperature is shown in Fig.2-b. The integrated intensity ratios of peaks are plotted at each temperature in Fig.2-c, and their pattern of change can be compared to the control peak (311 cm⁻¹). Upon cooling, all changes in the intensities of peaks in PTU were within the limits of change in the internal standard and were not persistent after cooling (Fig.2-b). This also applied to PTN and WO, except for the peak at 350 cm⁻¹ that changed differently after 70 °C and 50 °C, respectively, and did not recover after cooling. (Fig.2-b, c). In WG, many peaks changed differently than the control peak above 100 °C, however, only the peak located at 1670 cm⁻¹ did not recover after cooling. Most peaks in TPI changed similar to the control peak except the peaks at 350 cm⁻¹ and 2885 cm⁻¹ above 70 °C. The change was persistent in the peak at 350 cm⁻¹ only. Cooling STD to room temperature was associated with an increase in the intensity of most peaks. Changes were all

reversible except for the peaks at 1284 cm^{-1} and 1440 cm^{-1} , which exhibited changes above $80\text{ }^{\circ}\text{C}$ (Fig.2-c).

- Endodontic Sealers

The Raman and FT-IR spectra of endodontic sealers at different temperatures are plotted in Fig.4.

Heating the epoxy resin-based sealer above $100\text{ }^{\circ}\text{C}$ caused a noticeable drop in Raman peaks at 780 cm^{-1} , 1260 cm^{-1} , 2850 cm^{-1} , 2873 cm^{-1} , 2924 cm^{-1} , 3010 cm^{-1} and 3087 cm^{-1} (Fig.4-a). The intensities of peaks did not recuperate after cooling, which indicates irreversible changes. In the FT-IR spectra, bands located at 1293 cm^{-1} , 1233 cm^{-1} , 1362 cm^{-1} , 1503 cm^{-1} , $1650\text{--}2120\text{ cm}^{-1}$ all dropped gradually with heating but recovered after cooling. The peak at 900 cm^{-1} , however, dropped remarkably after $100\text{ }^{\circ}\text{C}$ and did not recover after cooling.

After cooling, the Raman (Fig.4-c) and FT-IR (Fig.4-d) spectra of the zinc oxide eugenol-based sealer were totally different than the spectra during heating. Raman peaks at 385 cm^{-1} , 480 cm^{-1} , 799 cm^{-1} , 1450 cm^{-1} , 1616 cm^{-1} , 1649 cm^{-1} , 2873 cm^{-1} and 2938 cm^{-1} dropped remarkably at temperatures above $125\text{ }^{\circ}\text{C}$, while peaks at 1151 cm^{-1} and 1190 cm^{-1} dropped above $150\text{ }^{\circ}\text{C}$. The FT-IR spectra showed a drop in the bands located at 740 cm^{-1} , 790 cm^{-1} , 1146 cm^{-1} , 1608 cm^{-1} , 1695 cm^{-1} after $100\text{ }^{\circ}\text{C}$, while new peaks appeared at 1400 cm^{-1} , 1430 cm^{-1} and 1536 cm^{-1} at the same temperature level.

Heating the premixed calcium silicate-based sealer (TotalFill) resulted in a drop in several peaks in its Raman and FT-IR spectra. However, all these changes were reversible and recovered upon cooling (Fig4-e, f). FT-IR peaks at 1972 cm^{-1} , 2026 cm^{-1} , 2335 cm^{-1} , 2362 cm^{-1} , 2550 cm^{-1} , and 3400 cm^{-1} dropped reversibly above $100\text{ }^{\circ}\text{C}$. Heating of BioRoot resulted in flattening in the FT-IR bands at 1650 cm^{-1} and 3400 cm^{-1} above $100\text{ }^{\circ}\text{C}$, which persisted after cooling. No changes were detectable in the Raman spectra however (Fig4-g, h).

Discussion

Although phase transformation of gutta-percha occurs at around $60\text{ }^{\circ}\text{C}$, many of the available heat carriers are programmed at $200\text{ }^{\circ}\text{C}$ or above. Conversely, the actual temperature achieved by heating devices was reported to be much lower than the reading displayed on their dial (Silver *et al.*1999,

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Venturi *et al.* 2002, Viapiana 2014). This discrepancy is confirmed by this study as the range of temperatures recorded was 33-110 °C although the devices were set at temperatures 180-230 °C (Fig.3). The maximum temperature level that was used during the Raman and FT-IR imaging was 200 °C, which is much higher than the actual temperature generated by most heat carriers. Such temperature levels are unfoundedly recommended by endodontic textbooks (Glickman & Walton 2009, Johnson *et al.* 2016) and hence it was used here. The duration of heat application is another factor that varies among clinicians. Prolonged heat application may endanger tissues and may affect the integrity of sealers (Atmeh & AlShwaimi 2017). Using heat carriers in continuous mode without automatic cut-out would risk prolonged heating and higher temperature levels (Fig.3-d, f).

It must be pointed out that the laboratory setup used to examine the chemical changes affecting tested root filling materials might be different than the clinical situation. The temperatures recorded in heat carriers were monitored at 37 °C, which may potentially change when the tips of the heat carriers are used inside a root canal. Despite data being available on the heat generated at the external root surface (Silver *et al.* 1999; Venturi *et al.* 2002, Viapiana *et al.* 2014, 2015), heat conducted to the inner root surface has not been well reported. A study measuring these temperatures in real-time during root canal filling (Donnermeyer *et al.* 2017) may not be representative as sealers would have come in contact with the thermocouples and affected their reading. Although using dentine as a substrate may seem more relevant to the clinical situation, sealers were examined on glass slides or metal plates, which was a necessity to evaluate the samples in the testing devices. However, this may not affect the results as changes affecting the root filling materials were being investigated while the heating tips were in direct contact with materials, which is the exact situation in the clinic. Acquiring data in real-time during heating meant that the samples were heated for longer durations in comparison with the situation in the clinic. However, the results reported here were comparable to previous results where short heating was used (Atmeh & AlShwaimi 2017).

Raman spectroscopy has been a useful method to study chemical changes in polymers, therefore it was valuable to investigate the impact of heat on root filling materials. However, Raman data must be interpreted cautiously, as several factors might confound the results (Robinson *et al.* 2014). Using an

internal standard as a reference to verify changes in the intensities of peaks is desirable (Michielsen 2001), henceforth two of stable constituents of gutta-percha were used; barium sulphate and zinc oxide (Mohazzabi & Seacry 1976). Raman spectra obtained for different gutta-percha brands confirmed their basic composition (Fig.2-d) (Freidman *et al.* 1975, Gurgel-Filho *et al.* 2003). Slight differences in their spectra may reflect differences in their molecular weight, crystallinity, and the percentage of their components (Goodman *et al.* 1974). This may explain variations in their thermal behaviour and response to heat (Fig.2-b) (Roberts *et al.* 2017).

Despite the different crystallographic structures of the α - and β -phases of gutta-percha, differences have been reported in their Raman spectra at 990 cm^{-1} , 1211 cm^{-1} , 2896 cm^{-1} , and 2914 cm^{-1} (Pathak *et al.* 2006). After cooling, the spectra of gutta-percha were similar to those before heating, which usually exists in β -phase (Fisher 1957, Pathak *et al.* 2007). Hence, no phase transformation may have occurred as no peaks of the α -phase were detectable, possibly due to the rapid cooling rate (Goodman *et al.* 1981). Changes in thermoplasticized (ITP) peaks at 2850 cm^{-1} and 2950 cm^{-1} , which represent symmetric and asymmetric stretching of the CH_2 respectively, were reversible (Fig.2-b). This may further support the unlikeliness of phase transformation (Pathak *et al.* 2006).

Examining the effect of heat on gutta-percha revealed irreversible change in the peak at 350 cm^{-1} in most brands (Figs.2-b, c). This peak represents the (C=C) bond located in the centre of gutta-percha's backbone (Pathak *et al.* 2006). The stretching mode of the same bond represented by the peak at 1670 cm^{-1} was also affected, which indicates that this bond is the most vulnerable upon heating and can be affected by temperature levels above 70 °C (Fig.2-b). This may agree with previous studies suggesting permanent alteration of gutta-percha due to chain cleavage after ageing or heating (Enoki *et al.* 2003, Silva *et al.* 2006). However, this cannot be correlated with oxidation, as Raman peaks representing C=O or C=O could not be detected. Thermal oxidation of gutta-percha was also suggested by Maniglia-Ferreira *et al.* (2008) producing (=C-H) bonds after consuming the (C=C) bonds in the polystyrene. Degradation of gutta-percha with heating was also reported using nuclear magnetic resonance imaging (Rodrigues *et al.* 2004), thermogravimetric analyses (Ferrante *et al.* 2011), X-ray diffraction analyses and differential scanning calorimetry (Maniglia-Ferreira *et al.*

2013). Hence, upon heating gutta-percha, the clinicians should be aware of the temperature levels that should be used to avoid inducing such changes (Table-2).

Some of the changes in the Raman and FT-IR spectra of the epoxy resin-based sealer could be due to the setting reaction and breaking of the epoxy rings (Ellis 1993) (Fig.4-a, b). The irreversible drop in the FT-IR band at 900 cm^{-1} (represents CO stretching in oxirane ring) may indicate accelerated setting of the sealer when heated above $100\text{ }^{\circ}\text{C}$ (Gonzalez *et al.* 2012). However, irreversible changes in the Raman peaks at 780 cm^{-1} , 2850 cm^{-1} , 2873 cm^{-1} , and 2924 cm^{-1} (represent the CH_2 group) can only be attributed to alterations in the structure of the resin. Such changes that appeared after heating the sealer above $100\text{ }^{\circ}\text{C}$ agree with what was reported before (Viapiana *et al.* 2015, Camilleri 2015, Atmeh & AlShwaimi 2017) suggesting irreversible damage to the sealer's backbone upon heating. Therefore, the temperature level should not exceed $100\text{ }^{\circ}\text{C}$ when epoxy resin-based sealers are used with warm vertical compaction (Table-2).

Zinc oxide eugenol-based sealers set through an acid-base reaction forming a hard matrix of zinc eugenolate (Henry *et al.* 1955). This explains the drop in the peak at 1190 cm^{-1} representing the COH group involved in the setting and the appearance of new peaks at 1350 cm^{-1} and 1500 cm^{-1} (Henry *et al.* 1955, Chowdhry *et al.* 2015, Khan *et al.* 2017) (Fig.4-c). Surprisingly, the spectra obtained after cooling the sealer differed from those obtained during heating (Fig.4-c, d). The Raman peaks that dropped after heating above $125\text{ }^{\circ}\text{C}$ represent bonds in eugenol's benzene ring, CH_3 and CO groups (Chowdhry *et al.* 2015). Hence, changes in these peaks could be due to molecular rearrangements in eugenol's structure that affects its stability and reactivity (McGraw *et al.* 1999, Turek & Stintzing 2013). Changes in the FT-IR spectra were detectable at a lower temperature ($100\text{ }^{\circ}\text{C}$), which may indicate even earlier changes. Although no changes were reported previously in this type of sealer (Viapiana *et al.* 2015), the present results clearly indicate irreversible changes upon heating and cooling, hence it may not be recommended to be used with warm compaction techniques (Table-2).

Heating calcium silicate-based sealers had been investigated previously suggesting no effect on their chemical composition (Viapiana *et al.* 2015, Camilleri 2015, Atmeh & AlShwaimi 2017). However, water loss upon heating was reported using FT-IR (Viapiana *et al.* 2015) and thermogravimetric analysis (Atmeh & AlShwaimi 2017), and hence FT-IR was used here to examine the sealers and

verify the Raman results and investigate the change in water content. The FT-IR was able to detect the irreversible drop of BioRoot's FT-IR peaks at 1650 cm^{-1} and 3400 cm^{-1} that represent vibrational modes of the OH group in water (Fig.4-h). This confirms water loss above $100\text{ }^{\circ}\text{C}$ through evaporation, which could not be reversed. The peak at 3400 cm^{-1} also dropped in TotalFill that contains no water but may contain propylene glycol (Fig.4-f). The drop was reversible, however. Due to the increasing background noise in the Raman spectra of Totalfill above $125\text{ }^{\circ}\text{C}$, it was difficult to recognise changes (Fig.4-e). However, the spectra obtained after cooling were very similar to those before heating, indicating that all the changes that occurred during heating up to $200\text{ }^{\circ}\text{C}$ were reversible. Further investigations about the effect of heat on the fluid uptake and integrity of calcium silicate based-sealers during heating are required considering their inorganic composition and nature, which render their setting based on water availability.

Conclusions

The results strongly advocate the importance of identifying the actual temperature levels of endodontic heat carriers by practitioners, and the suitability of sealers to be used at the temperature achieved. All tested heat carriers reached temperature levels below $60\text{ }^{\circ}\text{C}$ when used in cut-out mode, which is a safe level for most tested gutta-percha and all sealers. However, using some devices in continuous mode can produce higher temperature that may exceed the suggested safe levels. This may risk integrity of the root canal filling materials; hence clinicians should be cautious about heating durations. Zinc oxide-based sealers may not be recommended to be heated due to the detrimental effect of heat on its molecular structures. Tested epoxy resin and calcium silicate-based sealers bore heat below $100\text{ }^{\circ}\text{C}$, however higher temperature is not recommended.

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Conflict of Interest statement

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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References

- Arjunan V, Subramanian S, Mohan S (2001) Fourier transform infrared and Raman spectral analysis of trans-1, 4-polyisoprene. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **57**, 2547-54.
- Atmeh AR, AlShwaimi E (2017) The effect of heating time and temperature on epoxy resin and calcium silicate-based endodontic sealers. *Journal of Endodontics* **43**, 2112-8.
- Camilleri J (2015) Sealers and warm gutta-percha obturation techniques. *Journal of Endodontics* **41**, 72-8.
- Chowdhry BZ, Ryall JP, Dines TJ, Mendham AP (2015) Infrared and Raman spectroscopy of eugenol, isoeugenol, and methyl eugenol: conformational analysis and vibrational assignments from density functional theory calculations of the anharmonic fundamentals. *Journal of Physical Chemistry A* **119**, 11280-92.
- Dong ZW, Zhang CF, Deng H, You GJ, Qian SX (2006) Raman spectra of single micrometer-sized tubular ZnO. *Materials Chemistry and Physics* **99**, 160-3.
- Donnermeyer D, Schäfer E, Bürklein S (2018) Real-time intracanal temperature measurement during different obturation techniques. *Journal of Endodontics* **44**, 1832-6.
- Ellis B (1993) Introduction to the chemistry, synthesis, manufacture and characterization of epoxy resins. In: *Chemistry and Technology of Epoxy Resins* 1st edn; pp.1-36. Dordrecht, Netherlands: Springer.
- Enoki M, Doi Y, Iwata T (2003) Oxidative degradation of cis-and trans-1, 4-polyisoprenes and vulcanized natural rubber with enzyme-mediator systems. *Biomacromolecules* **4**, 314-20.
- Ferrante M, Trentini P, Croce F, Petrini M, Spoto G (2011) Thermal analysis of commercial gutta-percha. *Journal of Thermal Analysis and Calorimetry* **103**, 563-7.
- Fisher D (1953) Crystal structures of gutta-percha, *Proceeding of Physical Society B* **66**, 7.
- Friedman CM, Sandrik JL, Heuer MA, Rapp GW (1975) Composition and mechanical properties of gutta-percha endodontic points. *Journal of Dental Research* **54**, 921-5.
- Glickman GN, Walton RE (2009) Obturation, In: Torabinejad M, Walton RE, eds. *Endodontics; Principles and Practice*, 4th edn; pp. 298-321. St. Louis, MO, USA: Saunders Elsevier.

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González MG, Cabanelas JC, Baselga J (2012) Applications of FT-IR on epoxy resins-identification, monitoring the curing process, phase separation and water uptake. In: Theophile T, ed. *Infrared Spectroscopy-Materials Science, Engineering and Technology*, pp 261-84. London, UK: Intech Open.

Goodman A, Schilder H, Aldrich W (1981) The thermomechanical properties of gutta-percha. Part IV. A thermal profile of the warm gutta-percha packing procedure. *Oral Surgery, Oral Medicine, Oral Pathology* **51**, 544-51.

Goodman A, Schilder H, Aldrich W (1974) The thermomechanical properties of gutta-percha: II. The history and molecular chemistry of gutta-percha. *Oral Surgery, Oral Medicine, Oral Pathology* **37**, 954-61.

Gurgel-Filho ED, Feitosa JPA, Teixeira FB, de Paula RCM, Silva JBA, Souza-Filho FJ (2003) Chemical and X-ray analyses of five brands of dental gutta-percha cone. *International Endodontic Journal* **36**, 302-7.

Copeland Jr HI, Brauer GM, Sweeney WT, Forziati AF (1955) Setting reaction of zinc oxide and eugenol. *Journal of Research of the National Bureau of Standards* **55**,133-8.

Johnson, Kulid, Tay (2016) Obturation of the cleaned and shaped root canal system. In: Hargreaves KM, Berman LH eds. *Cohen's Pathways of the Pulp Expert Consult*, 11th edn; pp 280-323. St. Louis, MO, USA: Mosby.

Khan AS, Khalid H, Sarfraz Z, Khan M, Iqbal J, Muhammad N, Fareed MA, Rehman IU (2017) Vibrational spectroscopy of selective dental restorative materials. *Applied Spectroscopy Reviews* **52**, 507-40.

Lea CS., Apicella MJ, Mines P, Yancich PP, Parker MH (2005) Comparison of the obturation density of cold lateral compaction versus warm vertical compaction using the continuous wave of condensation technique. *Journal of Endodontics* **31**, 37-9.

Maniglia-Ferreira C, Bönecker G, Silva JB Jr, de Paula RC, Feitosa JP, Souza-Filho FJ (2008) Degradation of trans-polyisoprene after root filling with thermoplasticized techniques. *International Endodontic Journal* **41**, 296-302.

- Maniglia-Ferreira C, Gurgel-Filho ED, de Araújo Silva Jr JB, de Paula RCM, de Andrade Feitosa JP, de Sousa-Filho FJ (2013) Chemical composition and thermal behavior of five brands of thermoplasticized gutta-percha. *European Journal of Dentistry* **7**, 201-6.
- McGraw GW, Hemingway RW, Ingram LL, Canady CS, McGraw WB (1999) Thermal degradation of terpenes: camphene, Δ^3 -carene, limonene, and α -terpinene. *Environmental Science & Technology* **33**, 4029-33.
- Michielsen S (2001) Applications of Raman spectroscopy to organic fibers and films. In: Lewis IR, Edwards H eds. *Handbook of Raman spectroscopy: from the research laboratory to the process line*, 1st edn; pp.749-98. NY, USA: Marcel Dekker Inc.
- Mohazzabi P, Searcy AW (1976) Kinetics and thermodynamics of decomposition of barium sulphate. *Journal of the Chemical Society, Faraday Transactions 1: Physical Chemistry in Condensed Phases* **72**, 290-5.
- Ørstavik, DAG (2005) Materials used for root canal obturation: technical, biological and clinical testing. *Endodontic topics* **12**, 25-38.
- Pathak A, Agarwal R, Tandon P, Gupta VD (2007) Vibrational Dynamics and Heat Capacity of Trans-1, 4-Polyisoprene (α -Form). *Journal of Macromolecular Science, Part B: Physics*. **46**, 245-60.
- Pathak A, Saxena V, Tandon P Gupta, VD (2006) Vibrational dynamics of trans-1, 4-polyisoprene (β -form). *Polymer* **47**, 5154-60.
- Roberts HW, Kirkpatrick TC, Bergeron BE (2017) Thermal analysis and stability of commercially available endodontic obturation materials. *Clinical Oral Investigations* **21**, 2589-602.
- Robinson JW, Frame, Frame II (2014) *Undergraduate instrumental analysis 7th edn*. Boca Raton, FL, USA: CRC press.
- Rodrigues FHA, Santos EF, Feitosa JPA, Ricardo NMPS, Heatley F (2004) Ozonation of unstretched natural rubber film from Hevea brasiliensis studied by ozone consumption and ¹³C NMR. *Polymer International* **53**, 733-9.
- Schilder H, Goodman A, Aldrich W (1974) The thermomechanical properties of gutta-percha. 3. Determination of phase transition temperatures for gutta-percha. *Oral Surgery Oral Medicine Oral Pathology* **38**, 109-14.

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Schilder H, Goodman A, Aldrich W (1985) The thermomechanical properties of gutta-percha. Part V. Volume changes in bulk gutta-percha as a function of temperature and its relationship to molecular phase transformation. *Oral Surgery Oral Medicine Oral Pathology* **59**, 285-96.

Silva Jr JBA, de Paula RC, Feitosa JP, Gurgel-Filho ED, Maniglia-Ferreira C, de Souza Filho FJ (2006) In vivo aging of gutta-percha dental cone. *Journal of Applied Polymer Science* **100**, 4082-8

Silver GK, Love RM, Purton DG (1999) Comparison of two vertical condensation obturation techniques: Touch 'n Heat modified and System B. *International Endodontic Journal* **32**, 287-95.

Somers AE, Bastow TJ, Burgar MI, Forsyth M, Hill AJ (2000) Quantifying rubber degradation using NMR. *Polymer Degradation and stability* **70**,31-7.

Sun TL, Chen XM (2015) Raman spectra analysis for Ba [(Mg_{1-x} Ni_x)^{1/3}Nb_{2/3}] O₃ microwave dielectric ceramics. *AIP Advances* **5**, 17106.

Turek, C, Stintzing, FC (2013) Stability of essential oils: a review. *Comprehensive Reviews in Food Science and Food Safty* **12**, 40-53.

Venturi M, Pasquantonio G, Falconi M, Breschi L (2002) Temperature change within gutta-percha induced by the System-B Heat Source. *International Endodontic Journal* **35**, 740-6.

Viapiana R, Baluci CA, Tanomaru-Filho M, Camilleri J (2015) Investigation of chemical changes in sealers during application of the warm vertical compaction technique. *International Endodontic Journal* **48**, 16-27.

Viapiana R, Guerreiro-Tanomaru JM, Tanomaru-Filho M, Camilleri J (2014) Investigation of the effect of sealer use on the heat generated at the external root surface during root canal obturation using warm vertical compaction technique with System B heat source. *Journal of Endodontics* **40**, 555-61.

Wu MK, Van der Sluis LWM, Wesselink, PR (2002) A preliminary study of the percentage of gutta-percha-filled area in the apical canal filled with vertically compacted warm gutta-percha. *International Endodontic Journal* **35**, 527-35.

Figure legends

Figure 1: The experimental set up for heat measurements of heat carriers.

Figure 2: The effect of heat on different brands of gutta-percha: **(a)** Characteristic Raman spectra of different brands of gutta-percha with peaks of interest labelled according to the peaks in the table below **(d)**. **(b)** The percentage of change in the intensity of Raman peaks (labelled in the table (d)) when heated up to 200 °C and after cooling compared with the intensity at 30 °C. The solid and dashed lines represent the highest and lowest percentage of change for the control peak (311 cm⁻¹) respectively. **(c)** The effect of heat on the intensity of peaks of interest at each temperature level during heating gutta-percha from 30 °C to 200 °C. **PTU:** ProTaper Universal; **PTN:** ProTaper Next; **WO:** Wave One; **WG:** Wave One Gold; **TPI:** Thermo-plasticized Injectable; **STD:** Standardized gutta-percha.

Figure 3: Heat profiles of tested endodontic heat carriers and their specific set temperatures as indicated by the manufacturers. E&Q Master operated at 180 °C **(a)** and 230 °C **(b)** temperatures with automatic cut-out after 4s. Super Endo operated at 230 °C with a 4s cut-out **(c)** or in continuous mode for 30s **(d)**. System B operated at 200 °C with 4s cut-out mode **(e)** or continuous mode for 30s **(f)**. Heat profiles of the different tip diameters and tapers are shown for each device.

Figure 4: Real-time Raman and FT-IR spectra of different endodontic sealers obtained during heating from 25 °C to 200 °C (plotted from top to down) and after cooling (uppermost spectrum). **(a)(b)** Epoxy resin-based sealer (AH Plus). **(c)(d)** Zinc oxide eugenol-based sealer (Pulp Canal Sealer). **(e)(f)** Calcium silicate-based sealer (TotalFill BC Sealer. **(g)(h)** Calcium silicate-based sealer (BioRoot RCS). Peaks with changes in their intensity are marked with (*).

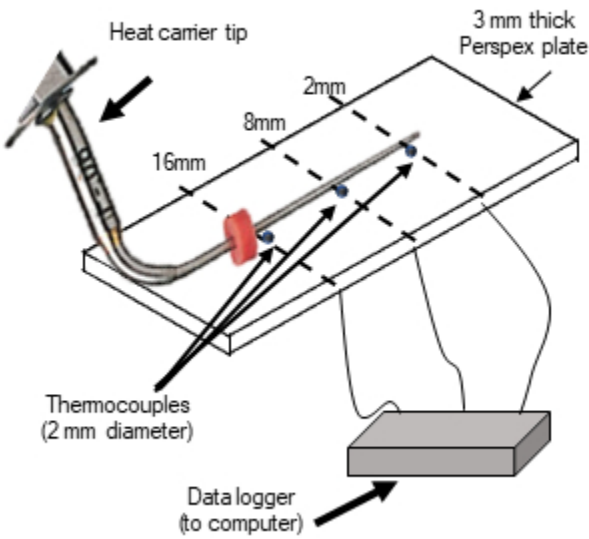
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Table 1 Safe temperature delivered by heat carriers:

Device	Manufacturer	Selected Temperature	Max. Temperature Delivered	Recommendation of use
E&Q Master	Meta Biomed	230 °C (Cut-out)	60 °C	- Heat generated does not exceed safe limit
SuperEndo B&L	B&L Biotech	230 °C (Cut-out) 230 °C (Cont.)	60°C 110 °C	- Heat generated does not exceed safe limit - Not to be used longer than 6 seconds
System-B	Sybrondental	200 °C (Cut-out) 200 °C (Cont.)	53 °C 66 °C	- Heat generated does not exceed safe limit

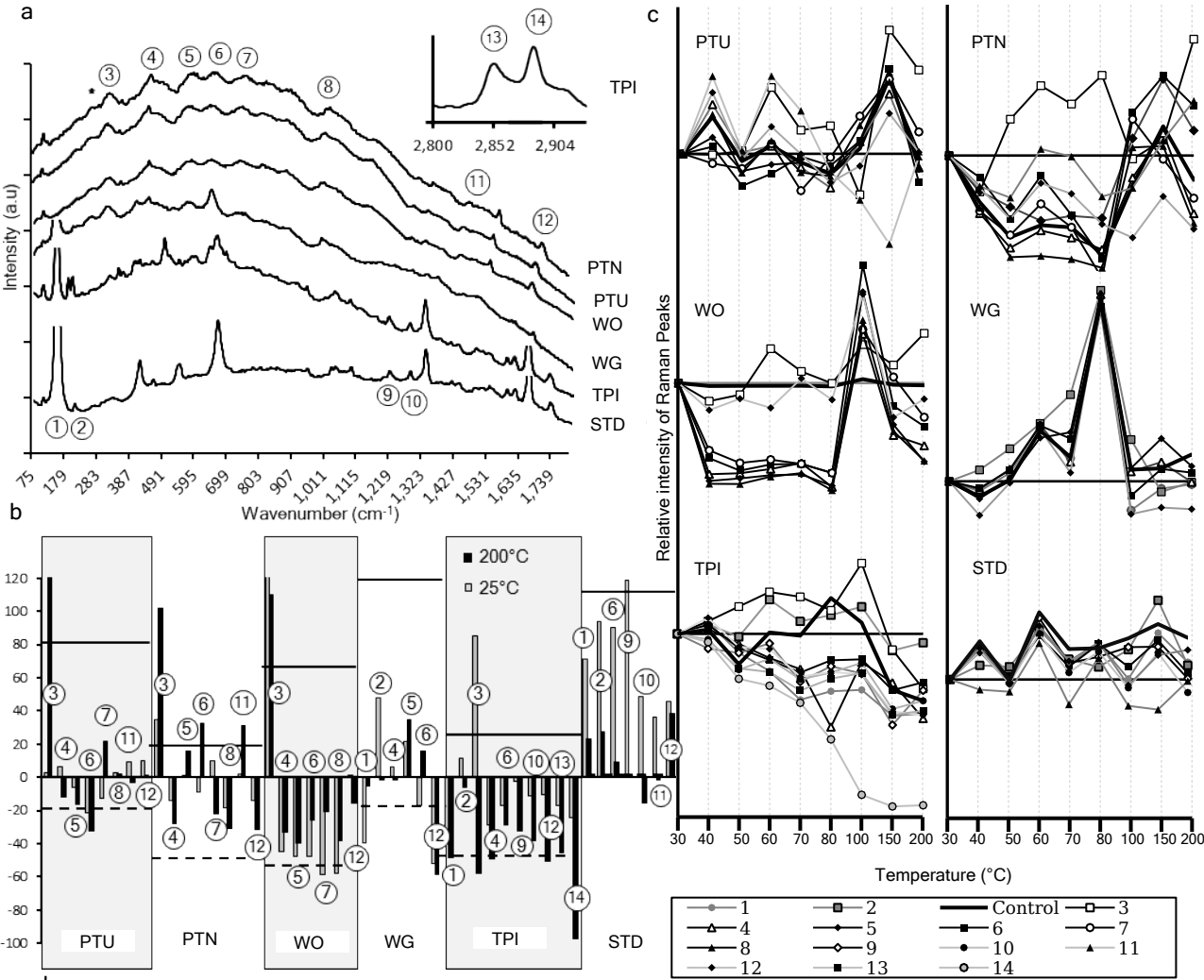
Table 2 Safe temperature limits for tested obturation materials during heating:

Material	Brand	Manufacturer	Safe heating temperature	Potential effects of heating beyond the safe temperature
Gutta-percha	ProTaper Universal	Dentsply	70 °C	Reversible changes above 70 °C
	ProTaper Next	Dentsply	70 °C	Irreversible changes involving gutta-percha's backbone (C=C bonds)
	Wave One	Dentsply	50 °C	Irreversible changes involving gutta-percha's backbone (C=C bonds)
	Wave One Gold	Dentsply	100 °C	Irreversible changes involving gutta-percha's backbone (C=C bonds)
	Thermoplasticized gutta-percha	Sure Dent	70 °C	Irreversible changes involving gutta-percha's backbone (C=C bonds)
	2% standardized points	Sure Dent	80 °C	Irreversible changes involving gutta-percha's backbone (CH ₂ group)
Sealers	AH Plus	Dentsply	100 °C	Accelerated setting & irreversible change in the resin's backbone (CH ₂ group)
	Pulp Canal Sealer	Kerr	Not recommended to heat	Changes detectable upon cooling, not recommended to be used with warm obturation
	TotalFill BC Sealer	FKG	100 °C	Reversible changes above 125 °C
	BioRoot RCS	Septodont	100 °C	Water loss above 100 °C



The experimental set up for heat measurements of heat carriers.

79x79mm (96 x 96 DPI)



¹Pathak et al. 2007; ²Arjunan et al. 2001; ³Pathak et al. 2006; ⁴Sun & Chen 2015; ⁵Dong et al. 2006.

